ISOLATION OF LIMONIN AND OBACUNONE FROM PHELLODENDRI CORTEX SHORTEN THE SLEEPING TIME INDUCED IN MICE BY α-CHLORALOSE-URETHANE

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Phellodendri cortex and its components, limonin and obacunone, shortened the sleeping time induced by α-chloralose and urethane (50 and 500 mg/kg respectively, intraperitoneal route) in mice.

KEYWORDS Phellodendri cortex; limonin; obacunone; berberine; sleeping time; α-chloralose-urethane; Rutaceae

The Chinese crude drug Phellodendri cortex (the bark of Phellodendron amurense RUPRECHT, Rutaceae) has been used as a stomachic. Berberine, a major component, has the pharmacological effects: anti-cholera toxin,[1] transient hypotensive,[2] cholinesterase inhibiting,[2] and potentiating pentbarbital hypnosis in mice,[3] etc. In the present study, we found that Phellodendri cortex and its components, limonin (1) and obacunone (2) shortened the sleeping time induced in mice by α-chloralose-urethane.

Powdered feed (Powdered Feed M, ORIENTAL YEAST CO., LTD.) containing 5% powdered Phellodendri cortex (P. cortex, UCHIDA WAKAN-YAKU) was fed to 4-week-old ddY male mice for 4, 7 or 11 days. During the administration period, feed was renewed every day, and water was available *ad libitum*. Each group consisted of 9 animals or more, and the body weights, within 10%, were used in the bioassay.

The results of bioassay shown in Table I were obtained as follows. After feeding for each test period, six groups (containing 3 groups of controls corresponding to each test period) were given α-chloralose (50 mg/kg) and urethane (500 mg/kg) intraperitoneally in saline. Sleeping time was calculated as the period from the loss of righting reflex till the time of recovery of righting reflex 3 times in a minute. The significance of the differences in sleeping time was calculated using Student's t-test.

P. cortex shortened the sleeping time at every administration period, and each treated group was significantly different from the controls. This indicates that the constituents in P. cortex may affect the central nervous system in mice. Further, the sleeping time was reduced in proportion to the length of the feeding periods. This effect is the opposite of the effect berberine has of lengthening the sleeping time induced in mice by pentobarbital.[3] In order to elucidate these results, we isolated the biologically active substances in P. bark using the bioassy, that is, the effect of shortening the sleeping time.

Table I. Effects of Phellodendri Cortex on Sleeping Time Induced by α-Chloralose and Urethane (50 and 500 mg/kg Respectively, Intraperitoneally)

Periods of feeding (d)	Body weight (g)	Sleeping time (min)	The number of animals	Reduction rate (%)
Control	23.6 ± 0.4	108.3 ± 9.1	10	
4	23.0 ± 0.2	78.5 ± 7.2*	12	28
Control	27.0 ± 0.5	94.7 ± 6.6	14	
7	27.2 ± 0.3	62.9 ± 5.2**	10	34
Control	30.9 ± 0.5	93.3 ± 11.6	10	
11	29.9 ± 0.5	38.1 ± 3.1**	9	59

Reduction rate (%) = (1 - sleeping time of treated group/sleeping time of control) x 100.

Each value represents the mean ± S.E.

* $p < 0.05$, ** $p < 0.01$, significantly different from control.

Average food intake of a mouse was ca. 4 g/day.

Powdered P. bark was extracted successively with normal hexane (n-hexane) and ethyl acetate (AcOEt) using a soxhlet apparatus. The residues at each extraction stage (He-residue and Ac-residue) were dried at 80 °C. Those residues were mixed with powdered feed to constitute 5% residue. The methods of administration and bioassay were as described above. Of 9 mice administered He-residue 5 did not lose the righting reflex. There was no significant difference between Ac-residue-treated mice and the controls in the sleeping time (Table II). This indicates that the active components occurred in AcOEt extract. AcOEt extract was re-extracted with 10% AcOEt in $CHCl_3$ at room temperature. After filtration, the residue contained berberine, and the filtrate was further separated into two fractions by silica gel column chromatography [eluent: AcOEt-$CHCl_3$]. The first fraction (47% yield) was active. This active fraction was subjected to repeated silica gel column chromatography to yield 1 and 2.[4] 1 and 2 were active components which shortened the sleeping time in a series of bioassays (Table II).

Since both 1 and 2 (ca 0.5 and 1.0% yield, respectively) in P. cortex had the same effect on the sleeping time, they may mutually potentiate the effect in feeding P. cortex. On the other hand, in the preliminary experiments, 0.1% berberine in powdered feed did not affect the sleeping time induced by α-chloralose-urethane,[5] and 5% P. cortex in powdered feed did not influence the sleeping time induced by pentobarbital.

Limonoids such as 1 and 2 are widely distributed in the edible Rutaceae plants, so limonoids may have some effects in humans. Since these limonoids may affect the sleeping time which is related to brain function, limonoids are important compounds to investigate in studying the mechanisms of hypnosis and the central nervous system in the brain.

Table II. Effects of Residues after Extraction with n-Hexane and AcOEt, Limonin, Obacunone and Other Components in 10% AcOEt-$CHCl_3$ Soluble Portion on Sleeping Time Induced by α-Chloralose and Urethane (50 and 500 mg/kg, Intraperitoneally)

Materials	Periods of feeding (d)	Body weight (g)	Sleeping time (min)	The number of animals	Reduction rate (%)
Control	11	31.4 ± 0.4	63.7 ± 5.3	9	
5% He-residue	11	29.9 ± 0.7	5 in 9 mice didn't lose the righting reflex		
Control	11	32.9 ± 0.3	68.3 ± 11.1	9	
5% Ac-residue	11	29.9 ± 0.5	60.7 ± 4.6	9	no difference
Control	9	29.0 ± 0.3	84.0 ± 6.0	10	
0.1% limonin	9	30.5 ± 0.4	52.0 ± 5.3**	11	38
0.1% obacunone	9	28.6 ± 0.4	62.6 ± 5.2**	10	25
0.1% others	9	31.1 ± 0.4	78.7 ± 9.5	10	no difference

Reduction rate (%) = (1 - sleeping time of treated group/sleeping time of control) x 100.

Each value represents the mean ± S.E.

** $p < 0.01$, significantly different from control.

Average food intake of a mouse was ca. 4 g/day.

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4) The physical properties and spectral data of obtained 1 and 2 agreed with those of authentic samples, respectively.

5) The sleeping time in the case of 0.1% berberine in powdered feed : 85.2 ± 6.7 min. The sleeping time in the case of control: 95.0 ± 11.4 min.

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